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Novel insights into the assessment of risk of upper gastrointestinal bleeding in decompensated cirrhotic children

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Abstract

Objectives: Cirrhotic children wait-listed for liver transplant are prone to bleeding from gastrointestinal varices. Grade 2-3 esophageal varices, red signs, and gastric varices are well-known risk factors. However, the involvement of hemostatic factors remains controversial because of the rebalanced state of coagulation during cirrhosis.

Methods: Children suffering from decompensated cirrhosis were prospectively included while being on waitlist. Portal hypertension was assessed by ultrasound and endoscopy. Coagulopathy was evaluated through conventional tests, thromboelastometry, and platelet function testing. The included children were followed up until liver transplantation, and all bleeding episodes were recorded. Children with or without bleeding were compared according to clinical, radiological, endoscopic, and biological parameters. In addition, validation of a predictive model for risk of variceal bleeding comprising of grade 2-3 esophageal varices, red spots, and fibrinogen level <150 mg/dL was applied on this cohort.

Results: Of 20 enrolled children, 6 had upper gastrointestinal bleeding. Significant differences were observed in fibrinogen level, adenosine diphosphate, and

Abbreviations: ADP, adenosine diphosphate; aPTT, activated partial thromboplastin time; ASPI, arachidonic acid; BA, biliary atresia; CFT, clotting formation time; CT, clotting time; EV, esophageal varices; GEV, gastroesophageal varices; INR, international normalized ratio; MCF, maximum clot firmness; ML, maximum lysis; NPV, negative predictive value; PPV, positive predictive value; Se, sensitivity; Sp, specificity; TRAP, thrombin receptor–activating peptide; TT, thrombin time; UGI, upper gastrointestinal bleeding.

thrombin-dependent platelet aggregation. The model used to compute the upper gastrointestinal bleeding risk had an estimated predictive performance of 81.0%. Platelet aggregation analysis addition improved the estimated predictive performance up to 89.0%.

Conclusions: We demonstrated an association between hemostatic factors and the upper gastrointestinal bleeding risk. A low fibrinogen level and platelet aggregation dysfunction may predict the risk of bleeding in children with decompensated cirrhosis. A predictive model is available to assess the upper gastrointestinal bleeding risk but needs further investigations.

Clinicaltrials.gov number: NCT03244332

KEYWORDS

chronic liver disease, esophageal varices, fibrinogen, hemostasis, platelet dysfunction, upper gastrointestinal bleeding

1 | INTRODUCTION

While awaiting a transplant, children suffering from decompensated cirrhosis run the risk of upper gastrointestinal bleeding because of ruptured esophageal/gastric varices, one of the most severe complications related to chronic liver disease.[1] Variceal bleeding shows a low mortality rate in children (less than 10%) compared to adults where it can reach up to 30%. However, it accounts for one-third of deaths in children with biliary atresia and having undergone Kasai portoenterostomy.[2] The risk stratification of bleeding is important to identify those who may benefit from a prophylactic treatment with band ligation or sclerotherapy. Up to now, there is no pediatric recommendation for primary prophylaxis with endoscopic ligation, sclerotherapy, or nonspecific beta-blockers in children.[3]

Although grade 2-3 esophageal varices and red marks on varices and gastric varices are known risk factors of variceal bleeding,[3,4] the role of hemostatic factors is still controversial. In cirrhosis, there are pro-hemostatic and anti-hemostatic alterations because of progressive hepatocellular failure, which concern the primary hemostasis, coagulation cascade, and fibrinolysis. This has been attributed to a rebalanced state of hemostasis in cirrhosis. This restored balance is delicate and more instable, likely to generate either a hemorrhagic tendency because of associated diseases (vitamin K deficiency in cholestatic liver disease, renal dysfunction, septicemia, etc.) or, and especially in adults, thrombotic complications.[5-8]

A retrospective study by Wanty et al[9] had brought to light the fact that a low level of fibrinogen (<150 mg/dL) is associated with a greater risk of upper gastrointestinal bleeding along with grade 2-3 esophageal varices and red spots. Based on these three features, a predictive model for the risk of variceal bleeding has been built. Moreover, further investigations into the role of hemostatic factors in the risk of upper gastrointestinal bleeding among children with chronic liver disease (thromboelastometry, platelet function analysis) are still required. A better understanding of these factors may allow a better risk stratification and targeted primary prophylaxis or therapy.

2 | PATIENTS AND METHODS

This was a prospective observational study performed between September 2014 and June 2016. It received approval from the ethics committee of University Clinics of Saint-Luc. Written informed consents were obtained from parents of children included in the study. We also state that all authors had access to the study data, reviewed, and approved the final manuscript.

2.1 | Patients

As Saint-Luc University Clinics is a tertiary center in the field of pediatric liver transplantation, notably since the initiation of living donor–related liver transplantation program in 1993, most of our patients are referred from abroad (eg, Eastern Europe or Algeria).

This study included children suffering from decompensated cirrhosis/end-stage liver disease wait-listed for a liver transplant, irrespective of underlying etiology. There is no clear definition of "decompensated cirrhosis" in the pediatric population contrary to adults. For this study, we considered as "decompensated cirrhosis" children with clinical or biological signs of impaired liver synthesis (hypoalbuminemia, low factor V level, etc.) and nutritional deficiencies, refractory ascites, variceal bleeding, or hepatic encephalopathy.[10] Any child with thrombophilia or a known hemorrhagic disorder was excluded.

Each patient had a complete evaluation of portal hypertension and coagulopathy during their pretransplant assessment, just after inclusion. We used Doppler ultrasound and gastroscopy to assess portal hypertension. The severity and localization of varices were described using standard classifications.[11,12] For hemostasis assessment, aside from conventional tests of hemostasis (INR, aPTT, TT, level of fibrinogen) and measurement of coagulation factors, further investigations were conducted. Platelet aggregation was explored by the Multiplate Analyzer® (Roche Diagnostics, Rotkreuz, Switzerland) through the ADP, the ASPI, and the TRAP pathways. Global assessment of hemostasis was done by thromboelastography and ROTEM® analysis

(Tem, Munich, Deutschland). Both measure the viscoelastic properties of the whole blood and provide information on the dynamics of clot development, firmness, and dissolution. Unlike thromboelastography, ROTEM® analysis has a higher sensitivity and a greater diagnosis power because of its multiple channels with different reagents to detect abnormalities in several components involved in hemostasis.[13] Only EXTEM (tissue factor pathway) and FIBTEM (contribution of fibrinogen to clot formation) channels were used in this study (See Appendix 1, which mentions collected and analyzed data).

The primary end-point was occurrence of upper gastrointestinal bleeding in the form of hematemesis and/or melena during the pretransplant period. Patients who underwent hemorrhagic complication were treated according to the Belgian guidelines for upper gastrointestinal tract bleeding management.[14]

2.2 | Statistical analysis

In order to identify risk factors for bleeding, data from patients with and without gastrointestinal bleeding were compared with univariate analysis. Multivariate approach was not suitable for this clinical research because of the high degree of colinearity among the large number of variables.

Nonparametric tests were used in view of the cohort size. Mann-Whitney test was applied to the continuous variables, whereas Fisher's exact test was used for categorical variables. Given the large number of parameters, *p*-values were adjusted using the Benjamini-Hochberg procedure to control the false discovery rate.[15] Factors with a corrected *p*-value <0.05 were considered as significantly different between the two groups and regarded as potential risk factors for upper gastrointestinal bleeding. Spearman correlation was also applied to assess the statistical dependence between the rankings of two variables.

As previously described, a prior developed predictive model of bleeding based on three relevant features (grade 2-3 esophageal varices, red signs onto varices, and low level of fibrinogen) was applied on our prospective cohort, as a part of the validation study for this model.[9] These predictions were compared to the results obtained from our study with determination of Se, Sp, PPV, and NPV.

The data of 75 patients which were used to develop the prior model were pooled with the data of the 20 patients of our prospective study. We also added data from 7 compensated cirrhotic children ("control patients") followed only in pediatric hepatology consultations (See Appendix 2, which indicates data from compensated cirrhotic children). In order to assess the generalization performances of the model of bleeding, a resampling procedure was applied, where predictive models are learnt from 80% randomly sampled data and predict the bleeding response on the 20% remaining and unseen data. These predictions were then compared to the clinical history of patients in terms of upper gastrointestinal bleeding. This is repeated 200 times, and the confidence intervals were computed following Nadeau's method. The same approach was done adding two features to the previously described model: ADP- and thrombin-dependent platelet aggregation.

3 | RESULTS

3.1 | Patient demographics and clinical history

Twenty-one children suffering from decompensated cirrhosis were included over a 21-month period. Eighteen children had biliary atresia with or without Kasaï portoenterostomy, 1 had Alagille syndrome, 1 had Budd-Chiari syndrome, and 1 had a type 2 progressive familial intrahepatic cholestasis. The patient with Budd-Chiari syndrome had to be excluded because of the presence of an activated protein C resistance.

Each patient presented features of end-stage liver disease (poor weight gain, growth curve faltering, nutritional deficiencies, digital clubbing, abdominal distention, collateral vessels on abdominal wall, severe jaundice). Abdominal distension was linked to hepatosplenomegaly and ascites, which require dietary sodium restriction and the use of diuretics (spironolactone and/or furosemide). No accurate measure of the amount of ascites was done. Two patients had already bled from esophageal varices before their arrival in our institution. No patient was under beta-blockers nor had a prophylactic banding ligation/sclerotherapy. Any blood derivatives were delivered just before the inclusion.

Blood samples were collected just after inclusion. As the comprehensive assessment of hemostasis requires a large volume of blood, this makes blood collection in some of our patients difficult. Several data are thus missing because of this technical aspect. Some radiological data are also missing because several radiological reports were incomplete.

No patient showed clinical sign of sepsis at the time of the inclusion. We noticed moderate elevation of protein C-reactive (up to 73 mg/dL) in just one patient suffering from biliary atresia. Bacteriological testing was non-contributory, and blood cultures remained sterile. As there was a risk of cholangitis, the patient underwent a bi-antibiotherapy for 10 days and had a good clinical/biological course.

During the pretransplant period, six children developed an upper gastrointestinal tract hemorrhage and were included in the "bleeding group." Three patients had hematemesis with or without associated melena, whereas the other three patients had recurring episodes of melena. Two of them had already bled before their admission. Bleeding complications occurred within 1-18 days after the pretransplant assessment.

Endoscopic features of bleeding patients (n = 6) were the following: grade 1 EV (n = 2), grade 2 EV (n = 3), grade 3 EV (n = 0), grade 4 EV (n = 1), GEV (n = 0), red signs onto varices (n = 4), and hypertensive gastritis (n = 6). Among patients without upper gastrointestinal bleeding (n = 14), we observed these endoscopic features: EV (n = 10), grade 1 EV (n = 6), grade 2 EV (n = 2), grade 3 EV (n = 1), grade 4 EV (n = 1), GEV (n = 1), red signs onto varices (n = 4), and hypertensive gastritis (n = 12).

Among patients with hematemesis, one was managed with somatostatin administration and a second one required an endoscopic ligation banding. A third patient died from uncontrollable bleeding despite two sclerotherapy sessions and an attempt to balloon tamponade. An upper endoscopy was performed in the first twenty-four

hours after the bleeding in these patients. Concerning recurring melena episodes, spontaneous resolution was observed for one patient, whereas two required somatostatin. No upper endoscopy was performed after the bleeding in these patients. For these patients, other causes of upper gastrointestinal bleeding (eg, gastro-duodenal ulcer) cannot be excluded. Red blood cells were transfused when hemoglobin level was below 7 g/dL, and platelet transfusion was considered when platelet count was below 50 000/μL.

In the end, out of twenty patients, nineteen patients received a liver transplant in our institution (median waiting time: 41.5 days, range: 1-98 days).

As presented in the demography (Table 1), no significant statistical difference had been observed between the "bleeding group" and the "without bleeding group." Although no significant statistical difference was identified, patients with upper gastrointestinal bleeding had a far lower platelet count (median: 104 × 10e3/μL, range: 45 × 10e3/μL-330 × 10e3/μL) than those without bleeding (median: 219 × 10e3/μL, range: 60 × 10e3/μL-424 × 10e3/μL).

3.2 | Risk factors for upper gastrointestinal bleeding

Table 2 presents a comparison between the 6 patients of the "bleeding group" and the 14 patients of the "without bleeding group" based on the clinical, radiological, endoscopic, and laboratory data.

No significant difference was observed between the two groups for presence of grade 2-3 esophageal varices, red marks, gastro-esophageal varices, hypertensive gastritis, or waiting time before liver transplantation. However, significant differences ($p < 0.05$) were observed for the following hemostasis related factors (Figure 1): ADP–dependent platelet aggregation, thrombin-dependent (TRAP)

TABLE 1 Demographic and laboratory features

	Patients with UGI bleeding (median values)	Patients without UGI bleeding (median values)	
	N = 6	N = 14	*p*-value
Diagnosis			
BA	n = 5	n = 13	>0.05
Others	n = 1	n = 1	
Waiting times (d)	43 (1-50)	40 (1-98)	>0.05
Age (mo)	10 (6-129)	9 (4-31)	>0.05
Sex			
Boys	n = 2	n = 6	>0.05
Girls	n = 4	n = 8	
Height (cm)	69 (64-136)	69.3 (48-81)	>0.05
Weight (kg)	7.64 (6.1-33)	7.77 (6.05-11.5)	>0.05
Body mass index (kg/m^2)	15.5 (13.9-18.3)	16.3 (12.5-20.6)	>0.05
Waist circumference (cm)	49 (44.5-76)	47 (43-56)	>0.05
Arm circumference (cm)	12 (10.5-14)	11.75 (7.2-14)	>0.05
CRP (<5 mg/L)	3.8 (0.5-27)	3.7 (0.3-73)	>0.05
Hemoglobin (10.2-12.8 g/dL)	8.75 (8.1-10.3)	8.7 (6.8-12.8)	>0.05
White cells (4.9-12.9 × 10e3/μL)	11.1 (3.7-33.8)	11.1 (4.7-25.9)	>0.05
Platelets (189-403 × 10e3/μL)	104 (45-330)	219 (60-424)	>0.05
Albumin (3.8-5.4 g/dL)	2.90 (2.5-3.4)	2.75 (2.3-3.9)	>0.05
GOT (<40 U/L)	158.5 (120-1017)	198.5 (70-756)	>0.05
GPT (<45 U/L)	127 (51.0-389)	95.5 (25-403)	>0.05
GGT (8-61 U/L)	77 (35-207)	122 (41-425)	>0.05
Total bilirubin (0.3-1.2 mg/dL)	23.95 (5.1-37.7)	18.91 (6.9-31.9)	>0.05
Conjugated bilirubin (<0.3 mg/dL)	19.35 (3.8-32.1)	13.55 (6.0-23.4)	>0.05

UGI, upper gastrointestinal; BA, biliary atresia.

TABLE 2 Univariate analysis (Mann-Whitney/Fisher's exact test and Benjamini-Hochberg correction)

	Patients with UGI bleeding (median values)	Patients without UGI bleeding (median values)	
	N = 6	N = 14	Adjusted *p*-value
Hemostasis			
aPTT (24-39.2 s)	44 (33-88)	36 (30-51)	>0.05
PT (9.35-14.30 s)	20 (15-32)	17 (12-22)	>0.05
INR (0.8-1.2)	1.8 (1.3-2.8)	1.5 (1.0-2.0)	>0.05
Thrombin time (10-18 s)	22 (16-32)	18 (16-22)	>0.05
Fibrinogen (150-450 mg/dL)	109 (69-267)	257 (126-392)	<0.05
Factor V (70%-140%)	49 (34-65)	70 (40-108)	>0.05
Factor VIII (50%-150%)	212 (198-296)	236 (182-345)	>0.05
Antithrombin III (78%-130%)	25 (17-57)	36 (19-100)	>0.05
Protein C (70%-130%)	17 (5-37)	18 (10-47)	>0.05
Protein S (76%-146%)	101 (70-152)	121 (70-151)	>0.05
Factor VIII/Protein C	15 (6.3-40)	13 (5.4-34)	>0.05
von Willebrand Factor			
>200	n = 5	n = 10	>0.05
<ou = 200	n = 1	n = 0	
CT—Thromboelastogram (190-600 s)	579 (385-987)	523 (410-710)	>0.05
CFT—Thromboelastogram (61-260 s)	293 (175-777)	182 (97-310)	>0.05
MCF—Thromboelastogram (50-72 mm)	40 (34-57)	51 (40-66)	>0.05
ML—Thromboelastogram (<21%)	2.0 (0.0-13)	4.0 (0.0-8.0)	>0.05
MCF—FIBTEM (9-25 mm)	9.0 (5.0-16)	17 (6.0-30)	>0.05
CT—EXTEM (38-79 s)	64 (57-72)	52 (39-68)	<0.05
CFT—EXTEM (34-159 s)	191 (120-1449)	85 (46-394)	>0.05
MCF—EXTEM (50-72 mm)	45 (24-49)	58 (35-68)	>0.05
ML—EXTEM (<15%)	2.0 (0.0-9.0)	8.0 (0.0-11)	>0.05
Multiplate ADP (607-936 AU/min)	103 (89-176)	368 (126-781)	<0.05
Multiplate ASPI (205-1086 AU/min)	202 (121-315)	412 (154-980)	>0.05
Multiplate TRAP (868-1473 AU/min)	265 (160-291)	558 (288-989)	<0.05
Ultrasound			
Spleen size (cm)	11 (10-13)	9.3 (7.2-13)	>0.05
Portal flow direction			
Hepatopetal	n = 3	n = 5	>0.05
Hepatofugal	n = 2	n = 6	
Hepatic artery resistance index			
>1	n = 1	n = 3	>0.05
<ou = 1	n = 4	n = 9	
UGI endoscopy			
EV grade			
No EV	n = 0	n = 4	>0.05
Grade 1	n = 2	n = 6	
Grade 2-3-4	n = 4	n = 4	
Red signs			
Presence of red signs	n = 4	n = 4	>0.05
No red sign	n = 2	n = 6	

(Continues)

TABLE 2 (Continued)

	Patients with UGI bleeding (median values)	Patients without UGI bleeding (median values)	
	N = 6	N = 14	Adjusted *p*-value
Hypertensive gastritis			
Hypertensive gastritis	n = 6	n = 12	>0.05
No hypertensive gastritis	n = 0	n = 2	
GE Varices			
Presence of GEV	n = 0	n = 1	>0.05
No GEV	n = 6	n = 13	

UGI, upper gastrointestinal; aPTT, activated partial thromboplastin time; PT, prothrombin time; INR, international normalized ratio; CT, clotting time; CFT, clotting formation time; MCF, maximum clot firmness; ML, maximum of lysis; EV, esophageal varices; GEV, gastroesophageal varices.

platelet aggregation, level of fibrinogen, and the CT in EXTEM condition of ROTEM® analysis. Remarkably, though the clotting time among patients who developed upper gastrointestinal bleeding was higher (64 seconds vs 52 seconds), it continued to be within the normal range (38-79 seconds). Albumin level, factor V level, and INR were not statistically different between the two groups. No difference in von Willebrand factor level was observed. As shown in Table 3, increasing clotting time in EXTEM condition of ROTEM® analysis in our cohort is more correlated to a low level of fibrinogen ($r = -0.70$, $p = 0.004$) than to a low platelet count, a low platelet aggregation function, or even a low coagulation factor level. No correlation was found between INR and the newly discovered risk factors. We highlighted a great correlation between platelet function testing and platelet count (Multiplate ADP: $r = 0.87$, $p = 0.0001$; Multiplate TRAP: $r = 0.74$, $p = 0.014$), which suggests that platelet aggregation testing (Multiplate®) depends on the platelet count. Even if platelet count is not statistically different between the two groups, the lower platelet count in the bleeding group (median: 104 000/μL) could have influenced the results of platelet aggregation testing. Finally, we noticed a weak correlation between CRP- and ADP-dependent platelet aggregation ($r = 0.50$, $p = 0.049$).

Concerning fibrinogen levels, even after omitting the 2 patients who previously bled, fibrinogen levels were still significantly reduced ($p < 0.05$) in the "bleeding group" (See Appendix 3, which demonstrates that level of fibrinogen is a potential risk factor).

3.3 | Establishment of a predictive model for risk of upper gastrointestinal bleeding

The previous predictive model of upper gastrointestinal bleeding (based on grade 2-3-4 EV, red signs, and low level of fibrinogen) was applied on our prospective cohort.[9] The following results were obtained: Se: 66.7%; Sp: 90.5%; PPV: 66.7%; NPV: 90.5%; accuracy: 85.2%. These results tend to independently confirm the theoretical performances assessed by Wanty et al This model has thus a promising potential clinical use.

We formed a new cohort which included the 75 patients of Wanty et al's cohort, the 20 patients of our prospective cohort and 7 compensated cirrhotic patients who did not bleed (See Appendix 2, which indicates data from compensated cirrhotic children). After obtaining a new cohort of 102 patients, we re-trained a model and estimated its generalization performances (i.e, its performances on new previously unseen samples) using a 200-resampling procedure. The following results were obtained: Se: 66.4%; Sp: 93.4%; PPV: 75.3%; NPV: 92%; accuracy: 88.6%; estimated predictive performance: 81.0% (95% CI: [70.5; 91.6]).

Based on these results, a web application has been developed and is actually available on the following URL (http://hrs2c2.com/). After entering the EV grade, the presence or absence of red signs onto varices, and the level of fibrinogen, this program assesses whether a patient suffering from decompensated cirrhosis presents a significant risk of upper gastrointestinal bleeding.

When using these 3 variables with ADP-dependent platelet aggregation and thrombin-dependent platelet aggregation, the predictive performances were the following: area under curve: 89.0% (95% CI: [68;100]); Se: 79.5%; Sp: 77.1%; PPV: 56.4 %; NPV: 94.8 %; accuracy: 77.6%. The predictive performances remained stable even after decreasing the number of used variables (Multiplate ADP, Multiplate TRAP, and fibrinogen level).

4 | DISCUSSION

4.1 | Involvement of hemostasis

Our study highlighted in a prospective way that endoscopic features of varices alone may not predict upper gastrointestinal bleeding in decompensated cirrhotic children and that impairments in the hemostasis could be involved in this risk. Low levels of fibrinogen and platelet dysfunction are indeed associated with upper gastrointestinal bleeding in decompensated cirrhotic children.

Regarding fibrinogen deficiency, as two of the studied patients had a previous hematemesis, their fibrinogen level could also have been reduced as a result of consumptive coagulopathy and thus may have overestimated its involvement in the upper gastrointestinal bleeding risk. However, even after excluding these 2 patients, fibrinogen levels of upper gastrointestinal bleeding patients were

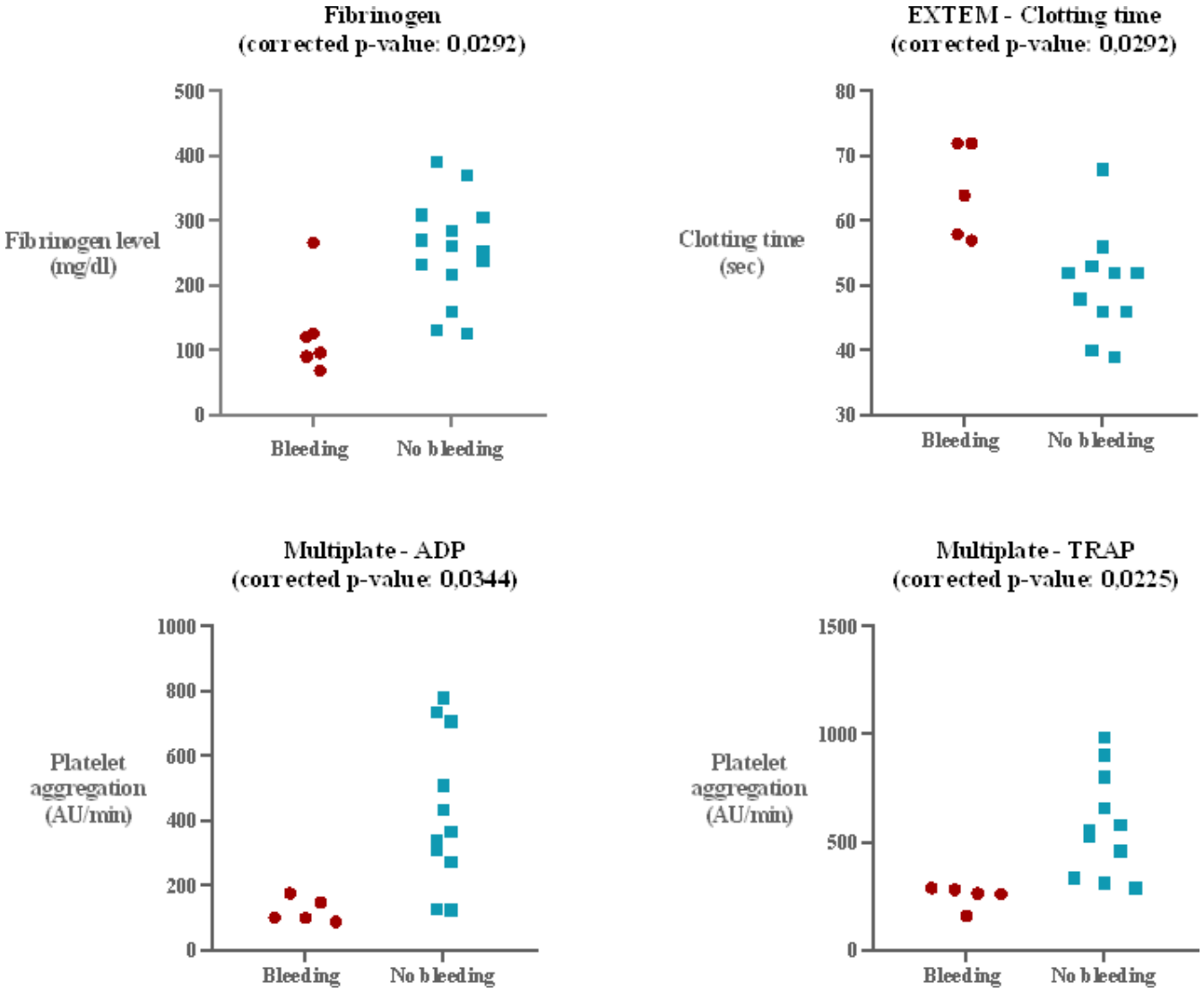

FIGURE 1 Risk factors for upper gastrointestinal bleeding. Scatter graphs for each highlighted risk factor of upper gastrointestinal bleeding (fibrinogen level, clotting time in EXTEM condition of ROTEM® analysis, adenosine diphosphate, and thrombin-dependent platelet aggregation) with patients of the "bleeding group" denoted by a point and patients of the "without bleeding group" denoted by a square. Mann-Whitney test and Benjamini-Hochberg correction were used as statistical analysis techniques with a corrected *p*-value <0.05

TABLE 3 Spearman correlation testing (*p*-value <0.05)

	Fibrinogen	Clotting time		Multiplate ADP		Multiplate TRAP
INR	$r = -0.56$	$r = 0.12$		$r = -0.24$		$r = -0.36$
	Fibrinogen	**Factor V**	**Platelet count**	**Multiplate ADP**	**Multiplate ASPI**	**Multiplate TRAP**
Clotting time EXTEM	$r = -0.69$ (*p*-value: 0.004)	$r = -0.30$	$r = -0.38$	$r = -0.41$	$r = -0.24$	$r = -0.43$
	Multiplate ADP			**Multiplate TRAP**		
Platelet count	$r = 0.87$ (*p*-value: <0.0001)			$r = 0.74$ (*p*-value: 0.014)		
Age	$r = -0.16$			$r = -0.11$		
CRP	$r = 0.50$ (*p*-value: 0.0487)			$r = 0.10$		

significantly reduced (See Appendix 3, which demonstrates that level of fibrinogen is a potential risk factor). We did not notice signs of sepsis which could interfere with fibrinogen level. These findings tend to confirm in a prospective way fibrinogen involvement in the upper gastrointestinal bleeding risk[9] and question the relevance of fibrinogen concentrates infusions in decompensated cirrhotic children.

Patients with decompensated cirrhosis frequently show abnormalities in laboratory tests reflecting primary hemostasis including platelet count or platelet aggregation tests. Thrombocytopenia is a common feature in chronic liver disease and is multifactorial (bone marrow suppression, altered thrombopoietin synthesis, hypersplenism linked to portal hypertension, platelet consumption, or platelet-associated antibodies).[16-18] At the present time, there is no consensus about the cutoff of platelets, which could be associated with a greater risk of upper gastrointestinal bleeding. Our study did not show any correlation between the platelet count and occurrence of upper gastrointestinal bleeding. Nevertheless, thrombocytopenia could have a negative impact for the management of these patients as it could enhance active bleeding from esophageal varices and bleeding after invasive procedures (eg, liver biopsy). Current guidelines from the American Association for the Study of Liver Diseases recommend delivering platelets when platelet count is less than 50 000-60 000/μL in case of active bleeding or before liver biopsy.[18] Decompensated cirrhosis is also characterized by a clear platelet aggregation dysfunction, demonstrated in vitro by platelet function testing and by flow cytometric analysis. Decreased responses after adenosine diphosphate, arachidonic acid, or thrombin stimulation have already been reported with Multiplate® analysis. The molecular mechanisms underlying this intrinsic hypoaggregability in cirrhosis seem to be multifactorial (reduced transmembrane signaling, storage pool defect in granules, upregulation of inhibitory pathways). Several plasmatic agonists (eg, von Willebrand factor) or antagonists (eg, high-density lipoprotein apolipoprotein E, fibrin degradation products, bile salts) could interact with platelet aggregation and enhance hypo- or hyperaggregability.[16-18] The contributions of these isolated findings are still a matter of discussion and are not fully understood. Another reported hypothesis for the qualitative platelet defect is an increased bacterial endotoxin bloodstream concentration and its consequent inflammation because of either the gut barrier disruption or the portosystemic shunting.[17] However, as mentioned above, we did not notice any sign of sepsis in our cohort of decompensated cirrhotic children. We just found a weak correlation between level of CRP- and adenosine diphosphate–dependent (ADP) platelet aggregation. Our study not only confirmed this platelet aggregation dysfunction but also its potential involvement in the upper gastrointestinal bleeding risk of decompensated cirrhotic children. Adenosine diphosphate–dependent (ADP) and thrombin-dependent platelet (TRAP) aggregation are indeed significantly reduced among children who had an upper gastrointestinal bleeding in our cohort. It should be noted that platelet aggregation tests are difficult to interpret because of their dependence on the platelet count. We found actually a great correlation between adenosine diphosphate–dependent (ADP) or thrombin-dependent (TRAP) platelet aggregation and platelet count. This hypoaggregability is currently a matter of discussion since recent data show many signs of platelet hyperactivation (eg, increased urinary excretion of thromboxane A2 metabolites, elevated level of soluble P-selectin). This discrepancy is difficult to explain but remains an area of interesting research.[18]

Thirdly, we brought to light in EXTEM condition of ROTEM® analysis that the clotting time seems to be a suitable parameter to assess the bleeding tendency. It is a dynamic parameter which depends above all on coagulation factor level including fibrinogen. Increased values are indeed observed among children with upper gastrointestinal bleeding.

Finally, we demonstrated the inadequacy of conventional coagulation tests, such as INR or aPTT, to predict an upper gastrointestinal bleeding in chronic liver diseases. As a dogma, the use of INR has been a common practice. Regardless of the characteristic prolonged values, they do not closely correlate with the bleeding events. Using this test could thus result in a misinterpretation of the dynamic balance of hemostasis and in an inappropriate administration of blood derivatives (such as fresh frozen plasma transfusions prior to procedures).[5-8]

4.2 | Portal hypertension involvement

With regard to portal hypertension involvement, grade 2-3 EV, presence of red signs onto varices, and gastric varices are undeniable risk factors of upper gastrointestinal bleeding.[2-4,9] Unfortunately, such associations have not been highlighted through our prospective study with univariate analysis. Among the 6 patients suffering from decompensated cirrhosis who had an upper gastrointestinal bleeding, 2 (33%) had only grade 1 varices and 2 (33%) had no red sign onto varices. Moreover, no bleeding episode was observed among 4 children of the 14 other patients who did not bleed (29%) unless a significant theoretical risk according to the grade of varices and the presence of red signs. Such results would suggest that portal hypertension is not the sole causal factor in the upper gastrointestinal bleeding risk.

4.3 | Primary prophylaxis of EV

There is a limited number of pediatric studies on primary prophylaxis efficacy and safety against EV bleeding in cirrhotic children.[4,19-21] Since its initiation at our institution in 1993, living donor–related liver transplantation has grown considerably and is now the main therapeutic option for children suffering from acute liver failure or decompensated cirrhosis. As a result, transplant waiting times is significantly reduced compared to non-living donor transplantation. This leads to the question whether prophylactic treatment of EV is required or not.

Trials conducted on pediatric patients suggested that sclerotherapy [22-25] and ligation banding [25,26] are relatively safe and could significantly reduce the risk of subsequent bleeding compared to a control group. Banding seems to require less sessions for a complete eradication of esophageal varices and to be responsible for fewer complications than sclerotherapy (stenosis, etc.).[27] It should therefore be considered as the best prophylactic option.[2] However, the size of the banding material is still a limiting factor. As fibrinogen level could be regarded as a potential risk factor for upper gastrointestinal bleeding, fibrinogen infusions would be a theoretical and non-invasive prophylactic option, although there is currently no proof that

fibrinogen injections improve the bleeding risk. Multicenter studies with a prospective design are required to confirm this hypothesis.

There are currently no guidelines to decide which patient would benefit from these procedures. In a previous retrospective study, we have built a predictive model of EV bleeding risk based on the endoscopic pattern (grade 2-3-4 EV and red signs onto varices) and low level of fibrinogen (<150 mg/dL).[9] We validated this model with a good accuracy (88.5%) and a good negative predictive value (92%) throughout our prospective study, and a Web application has been developed on the following website address (http://hrs2c2.com/). This would help pediatric hepatogastroenterologists to individualize decision management concerning primary prophylaxis of variceal bleeding in decompensated cirrhotic children. The results of this study cannot be extrapolated to a cohort of non-cirrhotic patients suffering from portal hypertension.

4.4 | Limitations

The most limiting factor of this study is the small number of included patients. Even if the size is considerable for decompensated cirrhosis children wait-listed for a graft, this cohort cannot allow us to draw any valid conclusions but only assumptions.

In addition, waiting times during the pretransplant period were different between some patients of this cohort. Therefore, some patients may have bled if the waiting times were longer.

5 | CONCLUSION

We demonstrated an association between hemostasis and the upper gastrointestinal bleeding risk in decompensated cirrhotic children. A low level of fibrinogen (<130 mg/dL) and platelet aggregation dysfunction could be regarded as risk factors along with grade 2-3 esophageal varices or red signs onto varices on the endoscopic examination. A predictive model of upper gastrointestinal bleeding risk is now available in order to select individual pediatric patient who will benefit from a primary prophylaxis of esophageal varices. Further investigations are nevertheless needed to confirm our findings and effectiveness of this model in clinical practice.

CONFLICT OF INTEREST

None declared.

AUTHORS CONTRIBUTIONS

Nicolas Bonnet: Conceived and designed the study; recruited patients; contributed to data acquisition; contributed to analysis and interpretation of data; performed statistical analysis; drafted the manuscript; and supervised the study. Xavier Stéphenne: Conceived and designed the study; recruited patients; contributed to data acquisition; contributed to analysis and interpretation of data; performed statistical analysis; critically revised the manuscript for important intellectual content; provided administrative, technical, and material support; and supervised the study. Jérôme Paul; Thibault Helleputte: Performed statistical analysis. Stéphane Eeckhoudt: Involved in acquisition of laboratory data and critically revised the manuscript for important intellectual content. Philippe Clapuyt; Renaud Menten; Dana Dumitriu: Involved in acquisition of radiological data and critically revised the manuscript for important intellectual content. Etienne Sokal; Françoise Smets; Isabelle Scheers; Sharat Varma; Francis Veyckemans; Thierry Pirotte; Caroline Prégardien; Cédric Hermans; Thierry Detaille; Raymond Reding: Critically revised the manuscript for important intellectual content.

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APPENDIX 1

Collected and analyzed data

Clinical	Body mass index, arm circumference, waist circumference, ascites, spleen size
Laboratory	Platelet count, albumin, total and conjugated bilirubin, liver enzymes
Hemostasis	INR, aPTT, thrombin time, fibrinogen, factor V, factor VIII, von Willebrand factor, antithrombin III, protein C, protein S, APC ratio
	Adenosine diphosphate, arachidonic acid, and thrombin-dependent platelet aggregation (Multiplate® analyzer)
	Clotting time, clotting formation time, maximum of clot firmness, maximum of lysis (Thromboelastogram and Rotem® analysis (EXTEM and FIBTEM))
Ultrasound	Ascites, direction of portal flow, hepatic artery resistance index
Endoscopy	Grade of esophageal varices, red signs, hypertensive gastropathy, gastric varices

INR, international normalized ratio; aPTT, activated partial thromboplastin time.

APPENDIX 2

Data from compensated cirrhotic children

	Median values
	n = 7
General	
Diagnosis	
BA	n = 6
Others	n = 1
CRP (<5 mg/L)	1 (0.1-7)
Hemoglobin (10.2-12.8 g/dL)	12.4 (9.6-15.8)
White cells (4.9-12.9 × 10e3/μL)	5.5 (2.8-7.9)
Platelet count (189-403 × 10e3/μL)	153.1 (69-303)
Albumin (3.8-5.4 g/dL)	3.8 (2.9-5)
GOT (U/L) (<40 U/L)	67.6 (21-133)
GPT (U/L) (<45 U/L)	58.9 (19-102)
GGT (U/L) (8-61 U/L)	124.7 (14-221)
Total bilirubin (0.3-1.2 mg/dL)	1.1 (0.3-8.8)
Conjugated bilirubin (<0.3 mg/dL)	0.2 (0.1-8.1)
Hemostasis	
aPTT (24-39.2 s)	33.6 (30-40)
PT (9.4-14.3 s)	12.7 (10.9-14.3)
INR (0.8-1.2)	1.1 (0.98-1.28)
Thrombin time (10-18 s)	16.1 (14-18)
Fibrinogen (150-450 mg/dL)	315.4 (249-419)
Factor V (70-140%)	91.1 (47-135)
Factor VIII (50-150%)	190.3 (119-236)
Antithrombin III (78-130%)	96 (62-112)
Protein C (70-130%)	55.3 (29-93)
Protein S (76-146%)	100 (60-212)
Factor VIII/protein C	4.2 (1.3-8.1)
von Willebrand factor	
>200	n = 3
<ou = 200	n = 4
CT—Thromboelastogram (190-600 s)	511.7 (178-831)
CFT—Thromboelastogram (61-260 s)	173.7 (24-249)
MCF—Thromboelastogram (50-72 mm)	52.4 (40-64)
ML—Thromboelastogram (<21%)	8.1 (1-11)
MCF—FIBTEM (9-25 mm)	13.6 (10-18)
CT—EXTEM (38-79 s)	53 (45-72)
CFT—EXTEM (34-159 s)	130.4 (77-206)
MCF—EXTEM (50-72 mm)	52 (40-62)
ML—EXTEM (<15%)	10.4 (5-18)
Multiplate ADP (607-936 AU/min)	447.4 (145-679)
Multiplate ASPI (205-1086 AU/min)	598 (158-1003)
Multiplate TRAP (868-1473 AU/min)	680.9 (223-1068)
Ultrasound	
Spleen size (cm)	14.4 (8-21.9)
Portal flow direction	
Hepatopetal	n = 7
Hepatofugal	n = 0
Hepatic artery resistance index	
>1	n = 0
<ou = 1	n = 7
UGI endoscopy	
EV grade	
No EV	n = 3
Grade 1	n = 4
Grade 2-3-4	n = 0
Red signs	
Presence of red signs	n = 0
No red sign	n = 7
Hypertensive gastritis	
Hypertensive gastritis	n = 1
No hypertensive gastritis	n = 6
GE varices	
Presence of GEV	n = 1
No GEV	n = 6

(Continues)

APPENDIX 2 (Continued)

BA, biliary atresia; aPTT, activated partial thromboplastin time; PT, prothrombin time; INR, international normalized ratio; CT, clotting time; CFT, clotting formation time; MCF, maximum of clot firmness; ML, maximum of lysis; UGI, upper gastrointestinal; EV, esophageal varices; GEV, gastroesophageal varices.

APPENDIX 3

Univariate analysis (Mann-Whitney test)

	Patients with UGI bleeding (median values)	Patients without UGI bleeding (median values)	*p*-value
	n = 4	n = 14	
Hemostasis			
Fibrinogen (150-450 mg/dL)	109 (91-267)	257 (126-392)	<0.05

UGI, upper gastrointestinal.

Fibrinogen levels from patients with and without gastrointestinal bleeding were compared with Mann-Whitney test. Contrary to Table 2, children who suffered from a previous hematemesis before their arrival in our institution were not taken into consideration so as not to overestimate fibrinogen involvement in the upper gastrointestinal bleeding risk.